

Exhibit H

Influence of Different Sling Materials on Connective Tissue Metabolism in Stress Urinary Incontinent Women

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Abstract: The aim of this study was to investigate the influence on the paraurethral connective tissue of different sling materials used in incontinence surgery. Biopsies from the paraurethral connective tissue were obtained intraoperatively from 16 women with stress urinary incontinence; all were operated on with the TVT procedure, 6 with Mersilene as the sling material and 10 with Prolene. Biopsies from 4 continent women with uterine bleeding irregularities, matched for age and parity, served as controls. New biopsies were obtained from all women after 2 years. The biopsies were examined histologically and analyzed for collagen concentration and solubility. An obvious inflammatory reaction with a significant increase in collagen extractability by pepsin was identified in patients where Mersilene was used as the sling material. A minimal inflammatory reaction without a significant change in collagen solubility was found in the Prolene group. In the control group no inflammatory reaction was seen. Mersilene gave rise to a significant foreign-body reaction in the paraurethral connective tissue after surgery. Such a reaction was not found with Prolene.

Keywords: Connective tissue metabolism; Stress urinary incontinence; TVT surgical procedure

Introduction

An effective closure of the female urethra in stress situations is considered to be dependent on an integrated action of various anatomical structures connected to the organ. Functionally the most important of these are the suburethral vaginal wall, the pubourethral ligaments, the pubococcygeus muscles and the paraurethral connective tissue [1,2]. There are reasons to believe that stress urinary incontinence in women is caused by defects in the aforementioned structures/tissues whereby the urethra cannot be closed in stress situations [3]. In all these structures connective tissue is an essential ingredient. We have previously found a changed biochemical composition of the connective tissue in supporting ligaments and the suburethral vaginal wall in stress urinary incontinent women [4–6]. Based on experimental investigations of the urethral closure mechanism, a new surgical procedure, the tension-free vaginal tape procedure (TVT), has been introduced into clinical practice. In the TVT operation a Prolene tape (Ethicon Inc., Somerville, New Jersey, USA) is used to support the midurethra. That continence has been obtained can be demonstrated intraoperatively by reconstruction of the different tissue defects [7]. It is crucial that the restored urethral support as induced by the TVT operation is maintained over time. By the same token, the implanted tape should not cause any adverse tissue reaction or rejection.

During the development of the TVT procedure different sling materials were used, such as Teflon, Gore-Tex (Gore, Flagstaff, AZ, USA) Mersilene (Ethicon Inc., Somerville, New Jersey, USA) and Marlex (Bard, Covington, GA, USA). All these materials caused a significant amount of tape rejection [2,8]. In a

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previous study Mersilene tape was found to induce a significant inflammatory reaction in paraurethral tissues, with a significant increase in collagen solubility by pepsin [8]. Such a reaction can explain the high number of sling rejections when using this tape/sling material.

The aim of this study was to compare tentative induced changes in the paraurethral connective tissue by polyester (Mersilene) and polypropylene (Prolene) used in TVT operations.

Materials and Methods

Transvaginal paraurethral punch biopsies were obtained from three groups of women. The first group comprised 6 women with stress urinary incontinence (SUI) operated on using Mersilene as the sling material. The second comprised 10 women with SUI operated on using Prolene, and a third comprised 4 continent women serving as controls. The biopsies were obtained at surgery for SUI for the two incontinence groups and at surgery for menorrhagia in the control group. New biopsies were obtained from all women 2 years later. All women after menopause had estrogen replacement therapy except for 2 who both were within 6 months of their last period. Except for SUI the incontinent women were healthy, had no ongoing medication, and no signs of infection or systemic disease. SUI was defined objectively by medical history, gynecologic examination, pad test and urodynamic examination. Clinical data are summarized in Table 1. All women gave their informed consent and the study was approved by the local ethics committee.

Table 1. Patient characteristics. Values are given as median (range)

	Mersilene	Prolene	Control group
Number	6	10	4
Age	58 (52–71)	61 (47–74)	55 (53–57)
Parity	2 (0–3)	2 (1–3)	2 (1–3)
Postmenopausal	5	9	2
Estrogen treatment	5	8	1

Procedure

Punch biopsies with a diameter of 6 mm were obtained at surgery 6–8 mm lateral to the external meatus of the urethra and to a depth of 10 mm. From all three groups new biopsies from the same place were obtained 2 years later. The actual area was chosen for reproducibility and because the quantity and density of the endopelvic fascia in this area is significant [3].

Collagen Analysis

The mean weight of the biopsies was 40 mg. Light-microscopic studies of the paraurethral biopsies confirmed that they consisted of connective tissue. The mucosa was carefully excised and the biopsy specimens were immediately immersed in liquid nitrogen and stored at -60°C until analyzed. The biopsies were extracted with 0.5 M Hac followed by digestion with pepsin, as described earlier [9]. The total collagen concentration was estimated as hydroxyproline, after hydrolysis in 6 M Hcl, according to Stegemann and Stadler [10].

Histology

To verify tentative histologic changes from the different sling materials light-microscopy examinations were carried out. The biopsies were fixed in formalin, dehydrated, and embedded in paraffin before sectioning and staining with Masson's trichrome.

Statistical Methods

Mean values \pm standard error of the mean were calculated. Student's paired *t*-test and the Mann–Whitney test were used to evaluate any differences between groups.

Results

A highly enhanced paraurethral collagen extractability was registered in stress urinary incontinent women after surgical treatment by the TVT procedure using Mersilene as tape material ($P<0.001$) (Fig. 1a). In this group two rejections of the tape occurred. In women operated on using Prolene mesh and in the control group no change in collagen extractability was found post-operatively ($P=0.82$ and $P=0.08$, respectively) (Fig. 1b, c). In addition, no tape rejection was found in the Prolene group. The total collagen concentration was unchanged in all three groups (Fig. 2a–c). The histologic findings in the connective tissue induced by the two different implanted tape materials are shown in Figs 3 and 4. As can be seen, there was a significant inflammatory reaction induced by the Mersilene mesh compared to the minimal reaction induced by the Prolene tape. Estrogen therapy had no influence on the outcome.

Discussion

In a previous paper we discussed the change in collagen extractability probably induced by the Mersilene tape at TVT operations. It was then suggested that the persistent change in collagen organization might be the result of recruiting new and more active fibroblasts around the tape [8]. Gradually it became clear that the change in

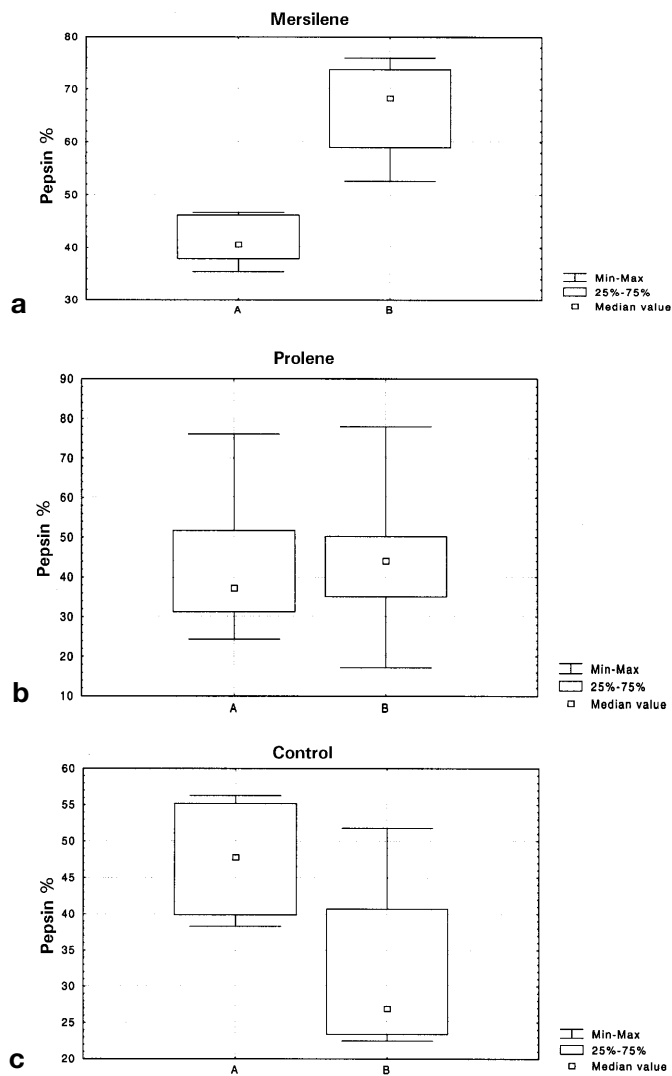


Fig. 1. Biopsies were obtained from the paraurethral connective tissue at surgery from incontinent women operated on with TVT using Mersilene (a), Prolene (b), and at surgery for menorrhagia from controls (c). New biopsies were obtained 2 years later. The specimens were extracted with acetic acid, followed by digestion with pepsin. The solubility, expressed as the amount of collagen extracted by pepsin digestion, is presented. (A) shows the solubility as pepsin% at surgery, and (B) 2 years later.

metabolism most probably reflects an ongoing tape rejection, rather than a proper fibroblast activation [11]. This is clearly illustrated in Fig. 4. As can be seen, there is a marked inflammation and fibrosis in the connective tissue around the Mersilene tape 2 years after its implantation. Inflammatory leukocytes infiltrate the site, triggered by chemotactic and activating mediators. This is followed by the mobilization of cytokines that directly and indirectly induce the proliferation of fibroblasts and endothelial cells [12]. It has been shown that fibrogenic cytokines can act directly to influence fibroblast activity and thus change the collagen extractability [13]. In the present study this is supported by the significant change in extractability by pepsin found in connective tissue in biopsies from patients

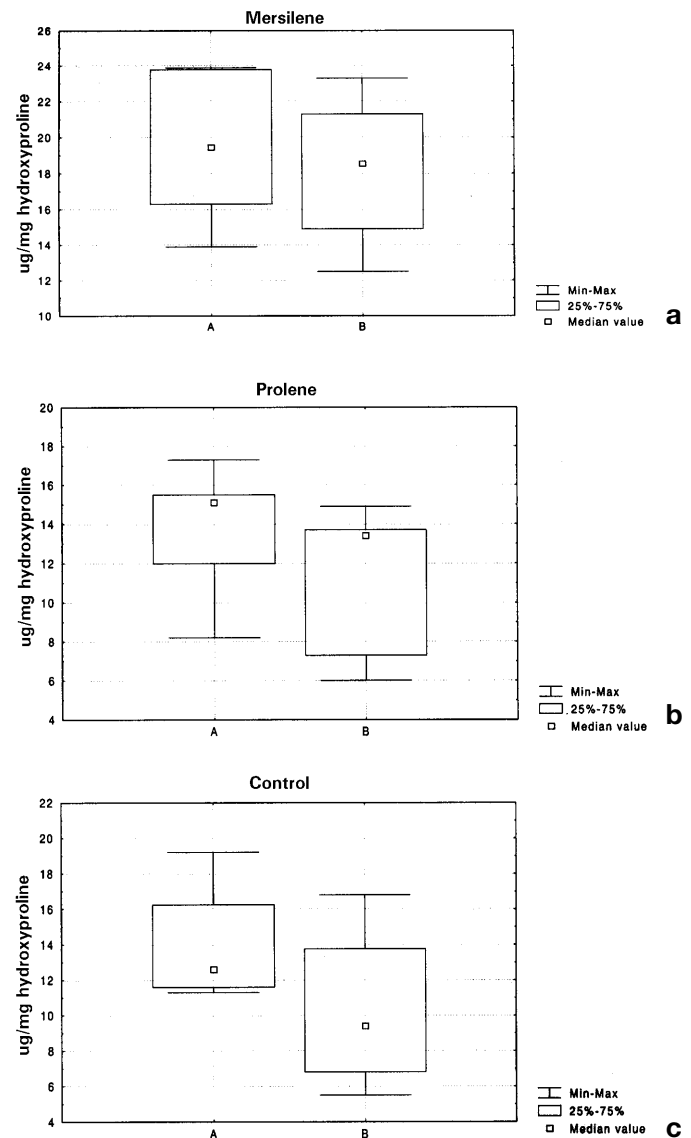


Fig. 2. Collagen concentration, measured as hydroxyproline, in biopsies from incontinent women operated on with TVT using Mersilene (a), Prolene (b), and at surgery for menorrhagia from controls (c). (A) shows the concentration at surgery, and (B) 2 years later.

operated on with Mersilene slings (Fig. 1). In contrast, there is practically no tissue reaction at all seen 2 years after TVT surgery when Prolene mesh was used (Fig. 3). In addition, no change in collagen extractability was found in the Prolene group (Fig. 1). There was no histological difference between paraurethral connective tissue in biopsies from patients operated on with Prolene tape and in controls 2 years after surgery. By the same token there was no statistical difference in collagen concentration or extractability. The diminutive decrease in both these parameters, as observed in the control group, might be the result of age, compensated for in the Prolene group by a slight stimulation of the fibroblasts by the foreign material. This observation is in agreement with the clinical experience from ~80 000 TVT

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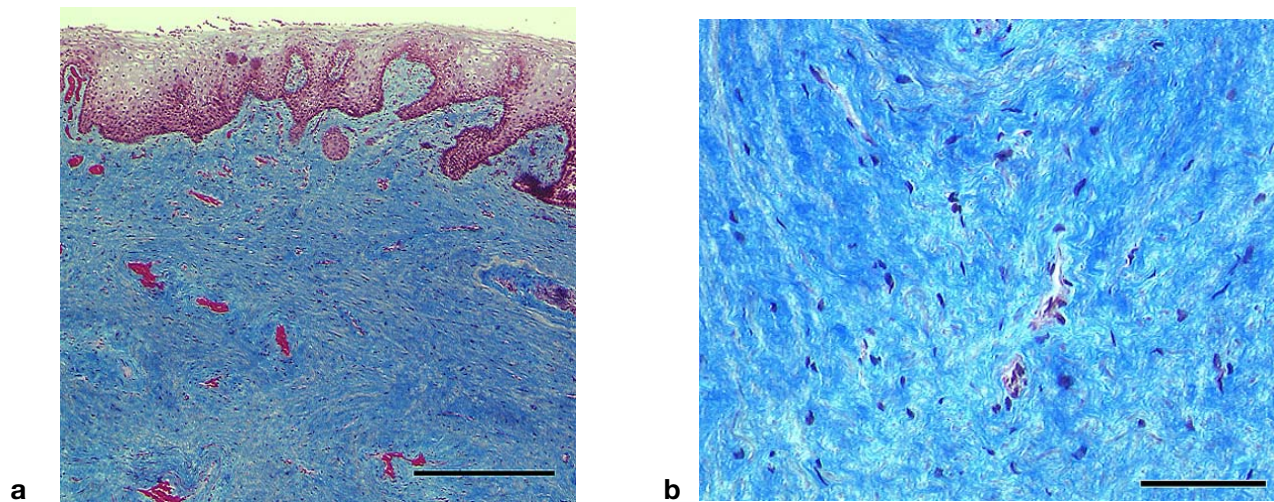


Fig. 3. a A specimen from the vaginal wall of a woman with stress incontinence 2 years after Prolene implantation. The squamous epithelium, stained greyish-red, is of a medium height, with some glycogen in the upper cell layers. There is no sign of inflammation in the epithelium. The underlying connective tissue (blue stained) is made up of densely packed collagen bundles. Numerous fibroblasts are seen scattered throughout the connective tissue. Blood vessels of different size are also noted in the connective tissue. There are no signs of fibrosis. Masson's trichrome. Bar = 500 μ m. **b** A higher magnification of the same specimen as in **a**. The connective tissue is made up of densely packed collagen bundles. Fibroblasts are scattered throughout the collagen. Some blood vessels are also seen. Masson's trichrome. Bar = 100 μ m.

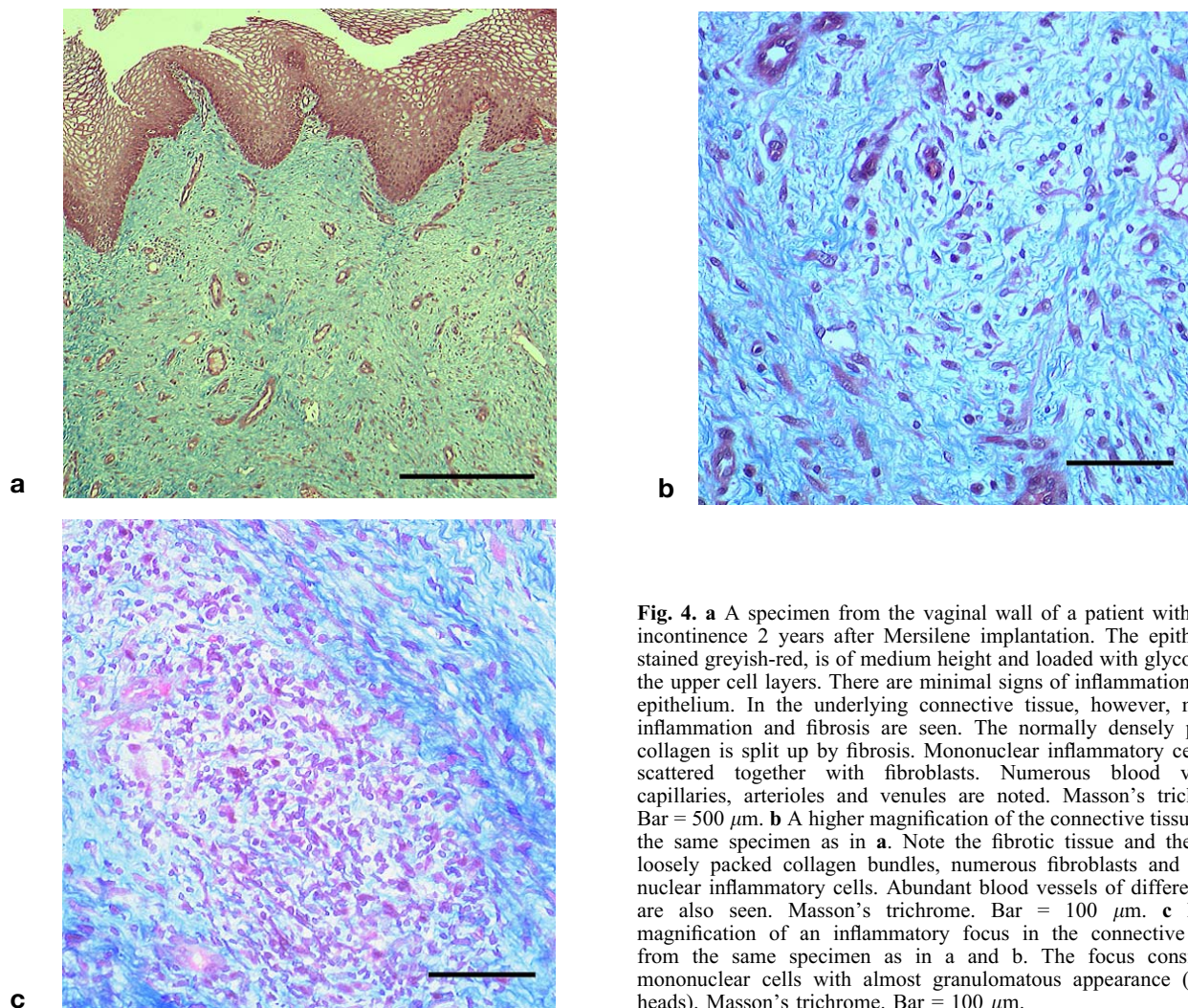


Fig. 4. a A specimen from the vaginal wall of a patient with stress incontinence 2 years after Mersilene implantation. The epithelium, stained greyish-red, is of medium height and loaded with glycogen in the upper cell layers. There are minimal signs of inflammation in the epithelium. In the underlying connective tissue, however, marked inflammation and fibrosis are seen. The normally densely packed collagen is split up by fibrosis. Mononuclear inflammatory cells are scattered together with fibroblasts. Numerous blood vessels, capillaries, arterioles and venules are noted. Masson's trichrome. Bar = 500 μ m. **b** A higher magnification of the connective tissue from the same specimen as in **a**. Note the fibrotic tissue and the more loosely packed collagen bundles, numerous fibroblasts and mononuclear inflammatory cells. Abundant blood vessels of different size are also seen. Masson's trichrome. Bar = 100 μ m. **c** Higher magnification of an inflammatory focus in the connective tissue from the same specimen as in **a** and **b**. The focus consists of mononuclear cells with almost granulomatous appearance (arrow-heads). Masson's trichrome. Bar = 100 μ m.

procedures using Prolene mesh with no tape rejections reported [14].

Conclusions

For obvious ethical and practical reasons the present study included a limited number of women only. Despite this, we consider the results to clearly indicate that the paraurethral connective tissue reacted differently to the two different sling/tape materials used for the TVT surgery. There was an increase in collagen extractability by pepsin combined with an obvious histologic inflammatory reaction after Mersilene tape implantation, indicating an increased risk of rejection of this sling material. In contrast, the Prolene tape did not induce such changes in the paraurethral connective tissue. The reports of no tape rejections after TVT operations with Prolene mesh support these experimental findings, suggesting Prolene mesh to be a suitable tape material for TVT surgery [14].

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